STUDY OF ANAESTHETICAL ACTIVITY OF ALKOXYPHENYLCARBAMIC ACID ESTERS USING STATISTICAL METHODS

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Abstract: Anaesthetical activity of 113 morpholinoethyl-, piperidinoethyl-, piperidinopropyl- and azepanoethyl- ester derivatives of alkoxyphenylcarbamic acid was characterized by several chemometrical techniques. The surface anaesthetical activity, *A*, and the infiltration anaesthetical activity, *B*, were correlated to lipophilicity, (expressed by the logarithm of the HPLC retention factor, log *k*), the length of the side alkoxy chain (represented by the number *n* of carbon atoms), molar mass *M* as well as the ester type. Principal component analysis and cluster analysis were used for predicting both types of the anaesthetic activity of the alkoxyphenylcarbamic acid esters.

Key words: alkoxyphenylcarbamic acid esters, anaesthetical activity, correlation analysis, cluster analysis, principal component analysis.

1. Introduction

The prediction of the compound property is the basic problem of the quantitative studies of the relationships structure - activity. The basic assumption is that the compounds with similar structure dispose of a similar biological activity (KUCHAŘ, REJHOLEC, 1987), as it was also stated in a similar anaesthetics study of HATRÍK *et al*., 1995). After discovery of a biologically active compound with a new structure, the next phase of the activity optimization is commonly characterized by varying the basic structure in order to achieve the maximal biological activity (HATRÍK *et al*., 1995a). The derivatives of basic ethyl and propyl esters of alkoxysubstituted phenylcarbamic acids have been prepared and studied as local anaesthetics due to their low toxicity (WAISSER *et al*., 2004). The HPLC capacity factor was used for characterization of the lipophilicity of the tested compounds (HATRÍK *et al*., 1995a). In this paper an attempt is made to correlate both types of the anaesthetical activity to the chemical shifts in the ^{1}H NMR and ^{13}C NMR spectra of the examinated compounds. When simulated chemical shifts are used then it is possible to omit laborious chemical synthesis – at least in the first phase of such study.

2. Experimental

The measured values of infiltration anaesthesia and surface anaesthesia were taken from the papers of HATRÍK *et al*. (1995), HATRÍK *et al*. (1995a) and HATRÍK *et al*. (1995b). The subject of the study was the data set comprising 48 compounds, for which both log *A* and log *B* were found, plus two data sets consisting of 62 and 68 compounds, for which log *A* and log *B* were acquired, respectively. All studied compounds were divided into 3 groups (categories) by increasing anaesthetical activity (the lower, middle and upper terciles). 19 variables describing molecule properties were used in our calculations: log *k* – the HPLC retention factor, *n* - number of carbon atoms on the side alkoxy chain, *M* – molar mass, *C*1, *C*2, *C*3, *C*4, *C*5, *C*6, *C*8, *C*10, *C*12 – the ^{13}C NMR chemical shifts of the corresponding carbons numbered in Fig. 1, *HCH*3, *H*1, *H*2, *H*12 - the ^{1}H NMR chemical shifts of the terminal methyl and the numbered protons (Fig. 1), respectively, *qH* – the quaternary nitrogen proton located in the ester ring. The mentioned nine ^{13}C and five ^{1}H NMR chemical shifts were simulated using the software package ACD Labs, ver. 7.0. The position numbering in Fig. 1 was also taken from ACD Labs. For basic data processing the MS Excel spreadsheets were used. The correlation analysis, cluster analysis and principal component analysis were performed using software packages JMP 7.0, SPSS 15.0 and STATGRAPHICS Plus 5.1.

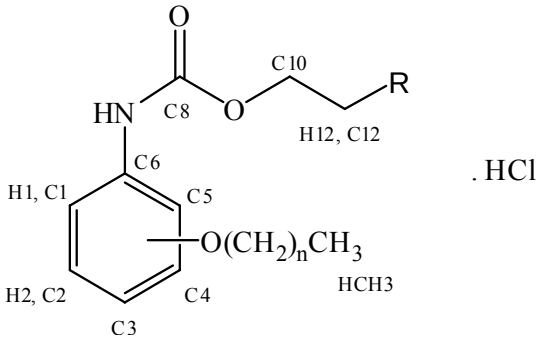

Fig. 1: Structure of the studied derivatives of alkoxyphenylcarbamic acid differing by *n* (*n* = 0 - 9), and the ester part R (R = morpholin-4-yl or piperidin-1-yl or azepan-1-yl).

3. Results and discussion

The *correlation analysis* shows the measure of correlation expressed by the pair (Pearson) correlation coefficients between all pairs of the studied variables. In the studied problem, the significant dependences of both log *A* and log *B* on the logarithm of the HPLC retention factor, log *k*, and mainly on *HCH*3 (^{1}H NMR methyl chemical shift) have been observed. In addition, further very significant dependences were discovered: log *A* – *n*, log *A* – *M*, and log *B* – *qH*. Some further simulated NMR chemical shifts correlate significantly with anaesthetical activity in the form of log *A* and/or log *B*. More details are summarized in Table 1.

Table 1. The pair correlation coefficients of the selected molecule properties with anaesthetical activity in the form of log *A* and log *B*, respectively.

Property	log *A*	log *B*	Property	log *A*	log *B*
log *k*	**0.4584**	**0.2064**	*C*8	-0.2300	0.0201
n	**0.5041**	0.1199	*C*10	0.1562	**-0.2967**
M	**0.5548**	0.0563	*C*12	-0.1589	0.1929
*C*1	-0.1309	-0.0593	*HCH*3	**-0.6062**	-0.1979
*C*2	0.1969	-0.1224	*H*1	0.1178	0.1304
*C*3	**-0.2910**	0.1266	*H*2	0.1166	-0.1283
*C*4	0.0416	-0.1298	H12	-0.1516	**0.2123**
*C*5	0.1164	0.1229	*qH*	-0.0162	**-0.3328**
*C*6	-0.0056	-0.1230			

Note: $R_{crit} = 0.2109$ ($n = 62$, $\alpha = 0.05$) for the dataset with log *A*; $R_{crit} = 0.2012$ ($n = 68$, $\alpha = 0.05$) for the for the dataset with log *B*.

Cluster analysis is represented by a dendrogram showing the distances among the objects (the studied compounds in this case) or the used variables (the selected properties of the molecule). The shorter distance in the dendrogram, the more similar are the clustered items. The results of cluster analysis depicted in Fig. 2 and Fig. 3 demonstrate that the shortest distance (expressed on the dendrogram ordinate) to the anaesthetical activity log *A* and therefore the largest similarity was found for log *k*, then for the number of alkoxy carbon atoms *n* and the molar mass *M* both for the squared Euclidean and the City-Block distances. The anaesthetical activity log *B* is most similar to *n*, then to log *k* and *C*1 if the squared Euclidean distances are used. When using the City-Block distances, the closest distance to the log *B* variable exhibit log *k* and *n*; then the next shortest distance exhibits *C*10.

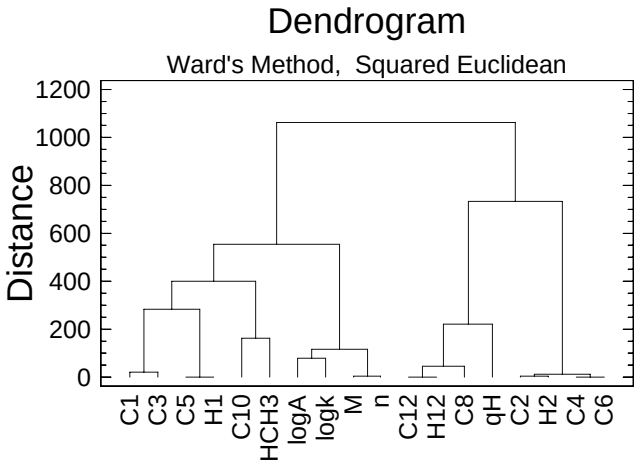

Fig. 2. Cluster analysis of 18 variables, 62 compounds, anaesthetical activity is expressed by log *A*. Software JMP 6.0. Ward's clustering method utilizing the Squared-Euclidean distances. The symbols used for describing the horizontal axis are introduced in experimental part.

It is noteworthy that the results of cluster analysis, both for log *A* as well as log *B*, are in good accordance with the results of the correlation analysis and confirm the previous results.

The *principal component analysis* was performed in the biplot form. The biplot describes the studied problem representing the used variables (molecule properties) by the rays and the objects (the studied compounds) by the object (compound) numbers or, alternatively, by the number of the category (lower, middle and upper) created according to the log *A* or log *B* measured values. When considering the variables, the most important mutual dependence exhibit those of them, which are represented by the rays forming an angle as close as possible to 0° or 180° (in such a case an inverse proportional dependence is supposed).

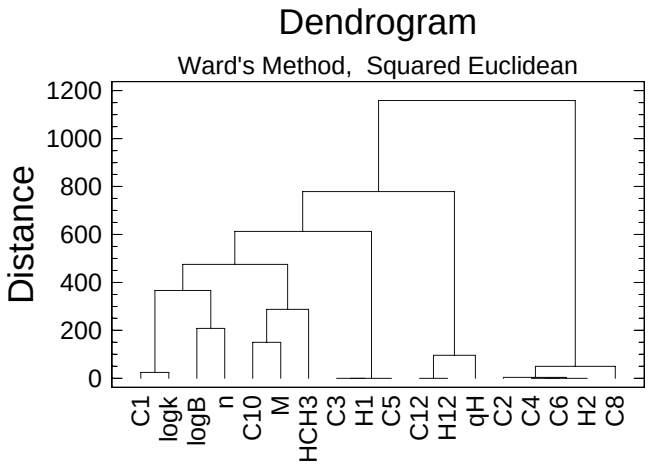

Fig. 3. Cluster analysis of 18 variables, 68 compounds, anaesthetical activity is expressed by log *B*. Software JMP 6.0. Ward's clustering method utilizing the Squared-Euclidean distances. The symbols used for describing the horizontal axis are introduced in experimental part.

The PCA results were received separately for the dataset with the log *A* measurements and that with log *B*. Neither log *A* nor log *B* was important with respect to the PC1 and PC2 variables but they were very important for the PC3. It was demonstrated by a long ray of the corresponding anaesthetical activity on the PC3-PC1 biplot, as it is visible in Fig. 4, calculated for the dataset with log *A*. In this figure, log *A* is closely correlated to *n*, *M* and then to log *k*. In addition, a very strong inverse dependence on the ^{1}H NMR chemical shift of the terminal CH_3 is observed. It was also found that the most correlated variables to log *B* are *n* and *M*, then log *k* and the ^{13}C NMR chemical shift of the carbon *C*1. Similarly to log *A*, a strong inverse correlation of log *B* with the ^{1}H NMR chemical shift of the terminal CH_3 was also observed.

It was also observable from the biplots calculated for both datasets that two groups of the studied compounds were clearly separated. By a detailed inspection of the dataset with log *A* it was found that one group is created mostly by ortho and para derivatives (the right group in Fig. 4) and another group is formed mainly by the meta

derivatives. The dataset with log *B* consists only from the ortho and meta derivatives, which (with some exceptions) formed two observed distinct groups.

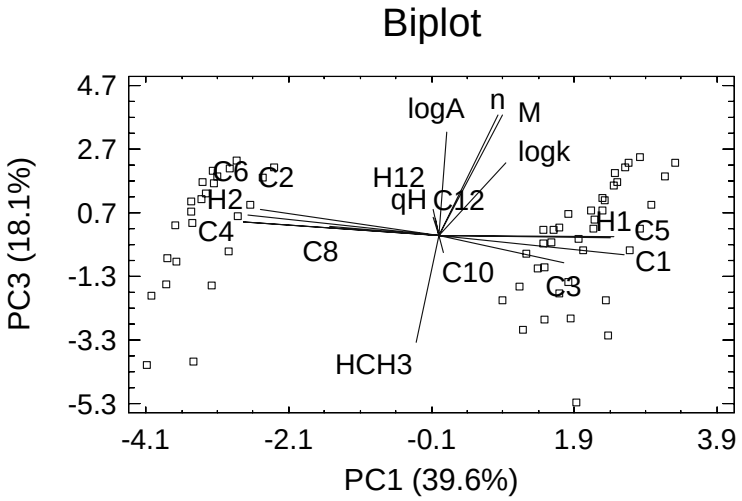

Fig. 4. Biplot PCA, 18 variables and 62 compounds. Software Statgraphics 5.1. Rays denote the used variables (molecule properties) and the symbols represent the studied compounds.

The PC3 axis can be assigned to anaesthetical activity – the higher PC3 the larger log *A* or log *B*. Since no one of the above mentioned two groups of compounds are located at higher or lower PC3 values it can be concluded that the position of the alkoxy chain is not a deciding factor determining any of the anaesthetical activity. Much more important is the length of the alkoxy chain, which is consistent with the larger molar mass and the HPLC retention factor so that a larger anaesthetical activity is predicted for a higher lipophilicity. The ^{1}H NMR chemical shift of the terminal CH_3 is inversely dependent on the length of the alkoxy chain (the distance from oxygen), which is in accord with the mentioned conclusions.

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